

# Detection of Ly-6B.2 Alloantigen in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)  
[3% Hydrogen Peroxide](#)  
[1% BSA Diluent](#)  
[Carezyme II \(Pepsin\)](#)  
[DAB Chromogen](#)  
[Hematoxylin](#)

### Blocking Serum: Normal Rabbit Serum

Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog# PK6104

### Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # SP-2001

### Primary Antibody: Rat Ly-6B.2 ALLOANTIGEN Monoclonal Antibody

AbD Serotec, Inc.  
Raleigh, NC 27604  
1-919-878-7978  
www.ab-direct.com  
Catalog # MCA771G  
Lot # 0911

### Negative Control Serum: Purified Rat IgG2a Isotype Control Serum

BD Biosciences  
San Jose, CA 95131  
www.bdbiosciences.com  
1-855-236-2772  
Catalog # 559073

### Secondary Antibody: Biotinylated Rabbit Anti-Rat IgG (H+L)

Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # BA-4001

Label Complex: R.T.U. Vectastain Elite ABC Reagent  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # PK-7100

### **Staining Procedure**

Positive Control Tissue: Endothelial cells  
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
4. Proteolytic-Induced Epitope Retrieval Using Pepsin  
Incubate the slides in Carezyme II: Pepsin (predilute) for 3 minutes at 37°C.  
(Allow the pepsin to reach room temperature before use.)  
Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.
5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
6. Block with 10% normal rabbit serum for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block for 15 minutes at room temperature.  
Quick rinse in 1X wash buffer.  
Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:500 dilution. Incubate for 1 hour at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute rat IgG2a control serum so that it's IgG2a protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the rabbit anti-rat secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

Exp. Date \_\_\_\_\_ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

*Updated 02/21/13*